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Published in:
Journal of Neuroscience Methods

DOI:
10.1016/j.jneumeth.2017.09.018

Publication date:
2018

Journal of Neuroscience Methods 293 (2018) 191–198

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Research article

Establishment of a chronic activity-based anorexia rat model

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HIGHLIGHTS

- Anorexia nervosa is a psychiatric disorder with a typically chronic course.
- The new activity-based anorexia rat modelis characterised by a fixed feeding regime and chronic low weight holding phase.
- This model enables the study of chronic starvation with initial hyperactivity and ensuring complete amenorrhoea.
- A 25% starvation level, early adolescent age and three weeks of starvation were demonstrated to be the best parameters.

ARTICLE INFO

Article history:
Received 7 June 2017
Received in revised form
28 September 2017
Accepted 28 September 2017
Available online 29 September 2017

Keywords:
Anorexia nervosa
Activity-based anorexia rat model
Acute vs. chronic starvation
Amenorrhoea

ABSTRACT

Background: Anorexia nervosa (AN) is often a chronic eating disorder characterised by body image disturbance and low body weight often associated with starvation-induced amenorrhoea and excessive exercise. Activity-based anorexia (ABA) is an animal model representing many somatic aspects of this psychiatric illness. We systematically manipulated the extent and length of starvation and animal age to find the optimal parameters to study chronic starvation.
New methods: Wistar rats had 24 h/day running wheel access and received 40% of their baseline food intake until a 20% or 25% weight reduction was reached (acute starvation). This body weight was then maintained for two weeks (chronic starvation). The rats of different ages of 4 or 8 weeks were used to represent early and late adolescent animals, respectively. The complete absence of a menstrual cycle was defined as the primary outcome parameter.
Results: Acute starvation caused a disruption of the oestrous cycle in 58% of the animals. During chronic starvation, a complete loss of the oestrous cycle could be found. Furthermore, 4-week-old rats exhibited higher levels of hyperactivity and amenorrhoea than 8-week-old animals. A 20% starvation level led to 90% loss of cycle, while a 25% starvation level triggered complete loss.
Comparison with existing methods: Most current ABA models focus on acute starvation, while most patients are chronically ill.
Conclusions: The optimal parameters to achieve complete amenorrhoea included early adolescence, chronic starvation and 25% weight loss. The new ABA model allows studying the effects of chronic AN on underlying behavioural, hormonal and brain pathobiology.

Abbreviations: ABA, activity-based anorexia; AN, Anorexia nervosa; GFAP, glial fibrillary acidic protein; NPY, neuropeptide Y; RWA, running wheel activity.

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https://doi.org/10.1016/j.jneumeth.2017.09.018
0165-0270/

1. Introduction

The third most common chronic disease in adolescence is anorexia nervosa (AN) (Gonzalez et al., 2007). Amenorrhoea is observed nearly ubiquitously in patients with AN, as it is seen as a reproductive precaution that prevents additional energy demands due to pregnancy in an already malnourished state (Herpertz-Dahlmann, 2015). The treatment success in patients with AN is limited (Espie and Eisler, 2015). At an average of 18 months after treatment, nearly 60% achieved a normal body weight; however, less than 60% of patients developed a normal cycle, and the risk for relapses was between 31 and 41% (Berends et al., 2016; Carter et al., 2004). After 7–10 years, Steinhausen reported that approximately half of AN patients recovered completely, 30% enhanced their condition and 20% of the patients stayed chronically ill (Steinhausen, 2009). Furthermore, the burden for patients, caregivers and costs for society are high (Schmidt et al., 2016). Therefore, research of the aetiology, sustaining factors and therapeutic options is urgently needed.

Activity-based anorexia (ABA) is the most commonly used animal model for studying AN. In 1967, Routtenberg and Kuznesof developed the original anorexia animal model using a restricted feeding schedule and access to running wheels (Routtenberg and Kuznesof, 1967). In 1953, Hall et al. demonstrated that rats were paradoxically hyperactive when food access was restricted, despite furthering their starvation by increasing energy consumption (Hall et al., 1953). Later, this model was called the ABA model (for a review see: Méquinion et al., 2015b). ABA mimics some of the core features of AN, including increased running activity, oestrous cycle disturbances and altered hormone production (Belmonte et al., 2016; Lee and Kinzig, 2017; Paré, 1977; Paulukat et al., 2016; Watanabe et al., 1992). The developing hyperactivity in ABA models can potentially be explained evolutionarily with a food seeking behaviour. This increased activity seems to be partially due to a lack of leptin, as it was shown that hyperactivity was significantly lessened when the rats were substituted with leptin (Exner et al., 2000). Hypoleptinemia is a known symptom of AN (Seitz et al., 2016). The ABA paradigm is well-established for analysing the neurobiological consequences after short-term starvation (Carrera et al., 2014). However, protocols for longer-term starvation that simulate chronic illness are scarce.

Other models of AN include a dehydration-induced rodent model (DIA), which included a hyperosmotic drink to induce dehydration (Reyes-Haro et al., 2016; Watts and Boyle, 2010). This model is performed for 4–14 days and characterised by a reduction in meal duration and weight loss (Callahan and Rinaman, 1998). However, changes in osmolarity in the blood and brain in this model are very high and can potentially influence research results, such as the difficulty of the brain to be able to distinguish between starvation and dehydration effects. Some groups mimicked AN with a mild food restriction in which 30–40% less food was given compared with the controls (Austad, 2001; Bi et al., 2003; Bruss et al., 2010; Hamrick et al., 2008; Yamamoto et al., 2009). Mild food restriction led to altered gene expression (e.g., increased arcuate NPY expression) and decreased leptin levels. However, these studies only examined short-term starvation lacking the hyperactive component, which is seen in 30–80% of the patients with AN (Herpertz-Dahlmann, 2015). However, ABA is the only model to combine (self-aggravated) food restriction and weight loss with hyperactivity. Most ABA protocols study short-term starvation ranging from 3 to 14 days (Méquinion et al., 2015b). In the original ABA model, the mortality rate was increased after chronic starvation, where the rodents continued to run during the feeding time instead of eating (Exner et al., 2000; Routtenberg and Kuznesof, 1967). Only one ABA study used a longer starvation period with free wheel access (Méquinion et al., 2015a).

The purpose of this study was to establish a modified ABA model that avoids the increased mortality rate of the original model while allowing for the analysis of the effects of chronic starvation, thus best representing the often chronically ill patient population. We modified the model of Mequinon et al. that used fixed amounts of food instead of time windows for feeding. However, instead of determining the fixed amounts of food given by comparing with the average of a control group, we calculated them individually by comparing with the same rat's food consumption during the ten days of acclimatisation. Furthermore, we preset a defined target weight rather than a reduction in quantity to increase control over the starvation process. This allowed us to reduce the variability of body weight and to minimise mortality within the treatment. We started with the acute starvation phase, where the animals received 40% of their previous food consumption until the target weight reduction of 20–25% body weight was achieved. Upon reaching this target weight, the food was adjusted daily to maintain this precise level of reduced body weight for another two weeks to mimic chronic starvation while insuring animal survival.

The second aim of this study was to optimise the new chronic ABA model in finding the optimal duration of starvation, adolescence or pre-adolescence, and level of weight reduction. Amenorrhoea was selected as a primary target parameter to eliminate the oestrous cycle as completely as possible to mimic this important clinical symptom in female AN and to obtain the maximum effect of gonadal suppression and hormonal change. As a secondary parameter, we selected the amount of running wheel activity (RWA) to be the seminal distinctive symptom of the ABA model. To find the best parameters, we tested acute (1 week) vs. chronic (3 weeks) starvation, 4 vs. 8-week-old rodents as well as a 20 and 25% extent of starvation. To minimise the distress of the rats, it was also important to determine the minimal necessary extent of starvation for complete amenorrhoea. We concentrated on female rats because most of the patients with AN are female (Herpertz-Dahlmann, 2015). However, the ABA model has also been shown to work in male rodents, but with gender differences in physical activity (Achamrah et al., 2017).

2. Materials and methods

2.1. Animals and care

Adolescent 4 and 8-week-old female Wistar rats (Charles River, Sulzfeld, Germany) maintained under a 12/12-h light/dark cycle (lights on at 7:00 am) were used in this study. The acute starvation group included one week of starvation, with the animals getting 40% of their average daily food intake during the acclimatisation phase until a 25% body weight loss was reached (Controls_acute_4W: n = 9; ABA_acute_4W25%: n = 9). Furthermore, the chronic ABA groups were subjected to an additional 2 weeks of starvation using different ages of rats, 4 and 8 weeks, and two different extents of starvation, 20 and 25% (Controls_chronic_4W: n = 12; ABA_chronic_4W20%: n = 6; ABA_chronic_4W25%: n = 12; Controls_chronic_8W: n = 12; ABA_chronic_8W20%: n = 6; and ABA_chronic_8W20%: n = 5). A schematic summary of the age, duration and extent of starvation and animal numbers in the different groups is shown in Fig. 1. The room was maintained at a constant temperature (21 ± 1 °C as the standard temperature). The facility was specifically pathogen free according to the FELASA Guidelines and certified according to DIN ISO 9001/2008.

2.2. Prearrangements of cages

Type IV polycarbonate cages (1820 cm^2, Polysulfone, Tecniplast GmbH) were provided with a cover and with an integrated run-

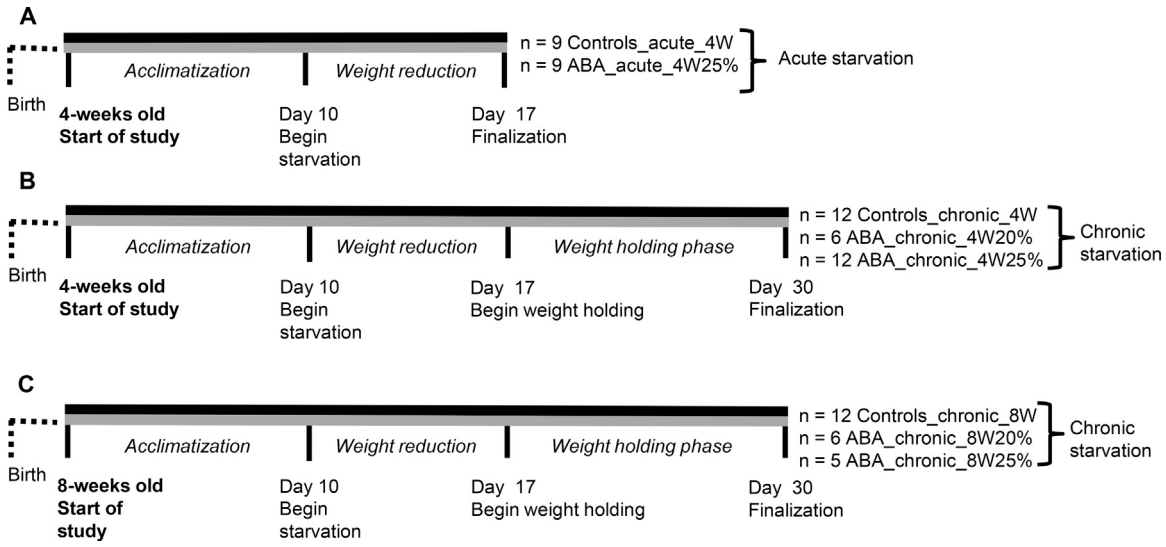

Fig. 1. The experimental protocol. A schematic summary of acute starvation (A), chronic starvation with 4-week-old animals (B) and chronic ABA with 8-week-old rats (C). The daily measurements of body weight, RWA and oestrous cycle were obtained.

ning wheel. Then, tachometers (BC 5.12, Sigma, Germany) were connected to the running wheels (circumference: 32.15 cm) by attaching the sensor to the outside metal of the wheels. The running wheels were cleaned using Antifect N liquid after each animal (propan-1-ol 35%, ethanol 23.5%). Furthermore, the cages for the rats were prepared with bedding, nestles, free access to water and the preset amounts of rat chow pellets (Harlan Teklad, cat. no. 2018S, USA or equivalent). The cages were tagged with identification papers.

2.3. Phase of acclimatisation

The female Wistar rats from Charles River were ordered, and on the first day, the rats were assigned to the different treatment groups. We examined up to twelve rats at the same time, and the average arrival weight was 88.32 ± 14.35 g for the 4-week-old rats and 196.28 ± 14.4 g for the 8-week-old animals. Each rat was housed individually in a running wheel cage. During the whole experiment, the body weight, food consumption, RWA and menstrual cycle were recorded daily at 12 pm. The rats were handled carefully to acclimate them to the daily handling procedure.

2.4. Estrous cycle determination

The menstrual cycle was determined by dropping NaCl on cotton buds, taking vaginal smears and wiping them carefully on glass slides. After drying for at least 30 min, the slides were stained with a Giemsa solution (Giemsa stock solution, ROTH T862.1, Germany). Following a fixation step in methanol (100%), the slides were incubated for 3 min in a 10% Giemsa solution and subsequently washed in tap water. The cell morphology was analysed under a microscope with 40× magnification, staging the approximately 4-day oestrous cycle in metoestrous, dioestrous, proestrous and oestrous stages. The incidence of the oestrous cycle was measured in 4-day blocks, as a usual rat cycle takes 4 days. Amenorrhoea was reached when the oestrous phase could no longer be found within a 4-day period (Dos Santos et al., 2011).

2.5. Acute starvation phase

To determine the daily amount of food to be given, first, the average daily food intake during the 10-day acclimatisation was calculated for each animal. After day 10, the modified ABA animals received only 40% of the baseline food intake until the target weight (80% or 75% of the outtake body weight on day 10) was reached. Thus, the ABA animals got the pre-weighed amount of food (60% food deprivation), while the controls continued to get *ad lib* food. All ABA animals received the entire amount of food at 12 pm. When the rodents reached the 20% or 25% weight reduction of their base weight, the acute starvation phase ended. The body weight, total RWA and oestrous cycle were recorded daily. The animals that received the acute starvation protocol were sacrificed after the starvation phase and further analysed. The animals that received the chronic ABA protocol continued with the chronic starvation period mentioned below.

2.6. Chronic starvation phase

On the first day of the weight holding phase, the amount of food was initially increased to 50–60% of the daily food intake during the acclimatisation phase. Then, the food was adjusted daily so that the rodents maintained their target weight. Every day of this phase, the body weight of the rats was determined and compared with the target weight. If the body weight deviated by more than 2.5%, the food was increased or reduced in increments of 5%. A separate control group was fed for the same time period but without starvation. At the end of chronic starvation, all animals were finalised for blood outtake, perfusion, brain tissue collection and further post-mortem analyses.

2.7. Distress of animals

The liability of rats was examined every day, including the appearance of coat and excrements as well as drinking behaviour. The load of distress was moderate after 20% starvation, while the starvation extent of 25% led to only slightly higher distress. Only a few of the 25% starved ABA animals showed small abnormalities, such as scrappy coat independent of starvation length. No animals had to be sacrificed earlier because of starvation-induced symptoms or distress.

2.8. Statistics

Data are represented as means and standard errors of the mean (SEM). The values for body weight and RWA of the acclimatisation

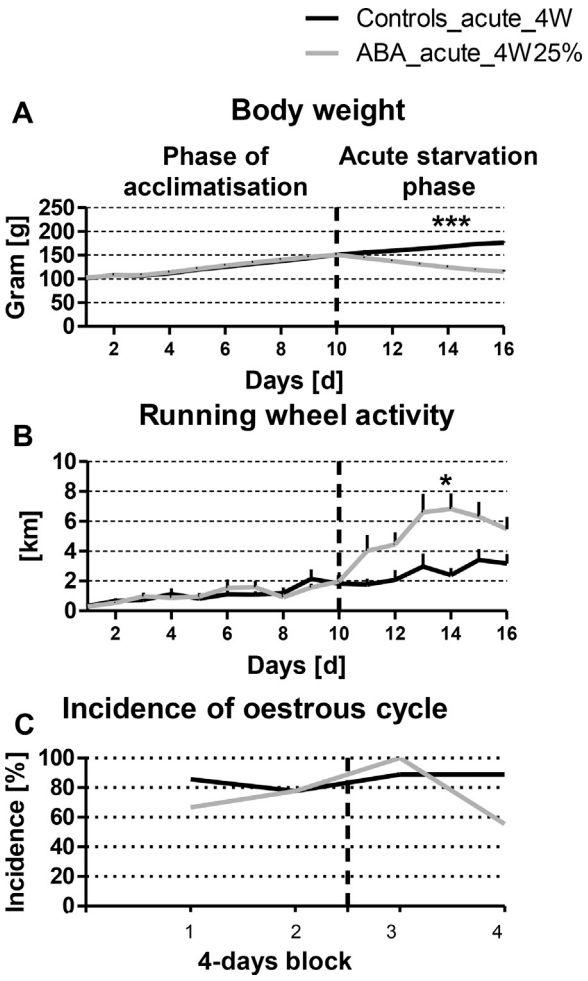

Fig. 2. The standardised body weight (A), running wheel activity (B) and incidence of oestrous cycle (C) during acute starvation in 4-week-old animals. The oestrous cycle was calculated in 4 day blocks mimicking the normal duration of cycle in rats, and chi-square test were performed. (A, B) $^{*}p \leq 0.05$, $^{***}p \leq 0.001$, two-way ANOVA with repeated measures. (C) Chi-square tests comparing oestrous cycle in ABA and controls.

phase were averaged between days 1–10, during the acute starvation phase on days 10–17 and during the body weight holding phase on days 17–30. The comparisons of the parameters body weight and RWA between the different subgroups of ABA animals and adequate controls within each phase of starvation were evaluated by two-way ANOVA with repeated measures with a significance level of 5%. Bonferroni's test was used for post-hoc comparisons between the different groups. The occurrence of the oestrous phase was tested within a 4-day period. Chi-squared tests were used to test if the oestrous phase in every 4-day block was significantly altered compared to the controls. All above analyses were conducted using SPSS version 20 for Windows (IBM, Chicago, IL, USA). To analyse the minimum group size needed to significantly differentiate between ABA animals and controls regarding amenorrhea, a power analysis was calculated using 80% power and a 5% alpha error (http://biomath.info/power/chsq.htm, chi square test on proportions).

3. Results

The primary outcome parameter to establish the best chronic ABA paradigm was the absence of any oestrous cycle as amenorrhoea, which, coupled with gonadal hormone suppression, is an important hallmark of AN. We studied acute starvation to obtain a 25% weight reduction in 4-week-old animals (Fig. 2). The body weight of both groups increased in the habituation phase because the rats were still adolescent and growing. At day 10, the starvation started, and the ABA rats lost 25% of their initial weight within 8 starvation days, while the control animals further increased their weight until the end of the experiment. About 90% of the animals showed a stable cycle at the beginning of starvation (88% of 4-week-old and 94% of 8-week-oldanimals). In the ABA_acute rats, there was only amenorrhoea in 58% of the animals; thus, the cycle was not completely disrupted yet (n = 9 Controls_acute, n = 9 ABA_acute, chi-square test, $p = 0.114$, $\chi(df) = 1$, $\chi^2 = 2.49$). The RWA of the ABA_acute group significantly increased to approximately 100% during the time of starvation (Controls_acute: 2.63 (SEM 0.63), ABA_acute: 5.62 (SEM 0.99), $p \leq 0.05$). The acute starvation ABA model was thus not useful for further experiments because only a portion of the animals developed amenorrhoea.

Our second experiment consisted of the chronic starvation protocol in 4-week-old animals with two different starvation levels, i.e., 20 and 25% (Fig. 3). As expected, the standardised body weight was decreased in both ABA groups compared with the respective controls during chronic starvation phase. Furthermore, the running behaviour of the 25% starved ABA animals was increased compared with the control group during the acute starvation phase, while for 20% animals no significant change was seen (n = 12 Controls_chronic: 2.28 (SEM 0.62) vs. n = 6 ABA_chronic_4W20%: 4.2 (SEM 0.83), 84%, $p = 0.29$; n = 11 ABA_chronic_4W25%: 5.78 (SEM 0.94), 156%, $p \leq 0.01$). This hyperactive behaviour in 25% ABA was also observed during the body weight holding phase (20% ABA: $p = 0.76$, 25% ABA: $p \leq 0.05$). At the end of the experiment, the animals in the 20% ABA and the 25% starvation group showed an approximate 80% loss and a complete loss of the oestrous cycle, respectively (chi-square tests: 20% ABA: $p \leq 0.001$, $\chi(df) = 1$, $\chi^2 = 13.85$; 25% ABA: $p \leq 0.001$, $\chi(df) = 1$, $\chi^2 = 23$). Thus, the chronic version of the model with a 25% starvation level seemed to be more suitable because 100% of these animals displayed amenorrhoea and hyperactivity was not observed in the 20% starvation level.

Next, we studied 8-week-old rats with two different starvation levels (20 and 25%, Fig. 4). As expected, these older rats showed a higher starting body weight than the younger animals, and significant reductions of this parameter were found in both ABA groups compared with the controls following the chronic starvation period. The running behaviour of the 20% ABA showed no significant differences with the controls during acute starvation (acute phase: n = 12 Controls_chronic_8W: 3.98 (SEM 0.94) n = 6 ABA_chronic_8W20%: 2.62 (SEM 0.52), −34%, $p = 1.00$, 25% ABA showed a trend towards hyperactivity during acute starvation (Controls_chronic_8W vs. n = 5 ABA_chronic_8W25%: 7.7 (SEM 2.56), 94%, $p = 0.07$). Furthermore, both ABA groups extended no statistically relevant differences in RWA during the chronic starvation phase (20% ABA: $p = 0.94$, 25% ABA: $p = 0.34$). The incidence of the oestrous cycle was significantly disrupted in both ABA groups by 40–50% but no real amenorrhoea was found (chi-square tests: 20% ABA: $p \leq 0.05$, $\chi(df) = 1$, $\chi^2 = 4.028$; 25% ABA: $p \leq 0.05$, $\chi(df) = 1$, $\chi^2 = 5.24$). Comparing early adolescent (4 weeks of age) and late adolescent (8 weeks of age) ABA rats, the younger 25% ABA animals thus developed hyperactivity during both starvation phases as well as complete amenorrhoea after the chronic experiment. The 8-week-old rats did not develop consistent amenorrhoea and developed no hyperactivity. The power analysis showed that at least 4 animals in each group are needed to significantly differentiate controls from 25% ABA 4-week-old animals after chronic starvation regarding amenorrhoea.

4. Discussion

We established a new modified ABA protocol that appears well suited to mimic the somatic effects of chronic starvation with-

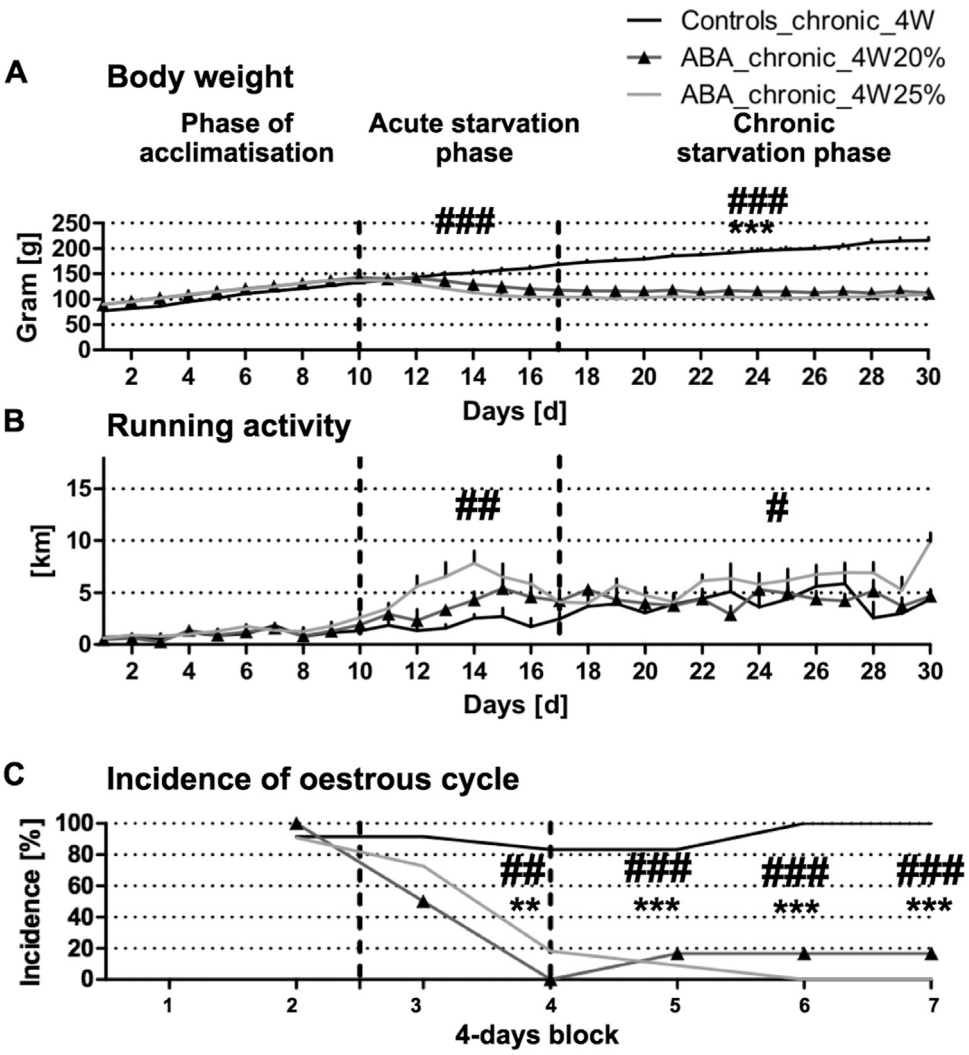

Fig. 3. The effects of the 20% and 25% extent of chronic starvation in 4-week-old ABA animals. The standardised body weight (A), running wheel activity (B) and incidence of oestrous cycle (C). (A, B) *Control vs. 20% ABA, **$p \leq 0.01$, ***$p \leq 0.001$, #Control vs. 25% ABA, # $p \leq 0.05$, ## $p \leq 0.01$, ### $p \leq 0.001$, two-way ANOVA with repeated measures. (C) Chi-square tests comparing ABA and controls.

out losing animals or unnecessary suffering. The optimal effects were achieved with 4-week-old animals that were 25% starved and maintained this starvation level for an additional 2-week phase. The model could induce somatic symptoms as seen in AN such as body weight loss, amenorrhoea and hyperactivity. Méquinion et al. (2015a,b) also studied a chronic ABA model in mice for 15–55 days, including 30% food restriction for 3 days and 50% for the rest of the experiment. However, they exclusively used adult mice, while the onset of the disease is mostly during adolescence, and they did not report on systematically varying age, starvation level and starvation length to establish optimal parameters for the model. Thus, our rat model appears to be optimised to balance important symptom induction with minimal animal suffering.

The incidence of the oestrous cycle was already partially disturbed (by 40–60%) following acute starvation in younger animals; however, chronic starvation seemed necessary for a complete loss of cycle. Dixon et al. reported a complete loss of cycle after 8 starvation days with 2 h/day *ad lib* food in Long-Evans rats (Dixon et al., 2003). Wistar rats, as used in our experiment, might thus be slightly less vulnerable to starvation than the Long-Evans rats. Furthermore, Dos Santos et al. showed that female Wistar rats with 50% food restriction for 7 days and access to a treadmill had a reduction in fertile phases but no complete amenorrhoea (Dos Santos et al., 2011). This result fits to our cycle measurements because in our acute starvation model (after seven starvation days), the cycle disturbance was also not complete. In addition, the study of Mequinion et al. showed complete absence of the oestrous cycle after 55 days (Méquinion et al., 2015a). Thus, amenorrhoea was mostly induced after chronic starvation protocols. In a study by Riddle et al., mice with caloric restriction but no running wheel access developed a reduction of the oestrous phases (no amenorrhoea), indicating that hyperactivity could stimulate the development of cycle absence (Riddle et al., 2013). Amenorrhoea was associated with oestrogen deficiency in the plasma of our modified ABA model, further underlining its effect on hormonal physiology (Paulukat et al., 2016).

Regarding the running behaviour, we demonstrated an increase in RWA during acute starvation, as expected in Wistar rats. However, RWA did not further increase during the body weight holding phase but remained at a stable and high level, potentially indicating an adaptation mechanism for survival, limiting the increased food seeking behaviour. Previous studies in humans and animals showed similar results (Barbarich-Marsteller et al., 2013; Holtkamp et al., 2006; Kanarek et al., 2009). In addition, Wu et al. found that ABA animals increased their RWA only for a few days, and then it resumed to the control level (Wu et al., 2014).

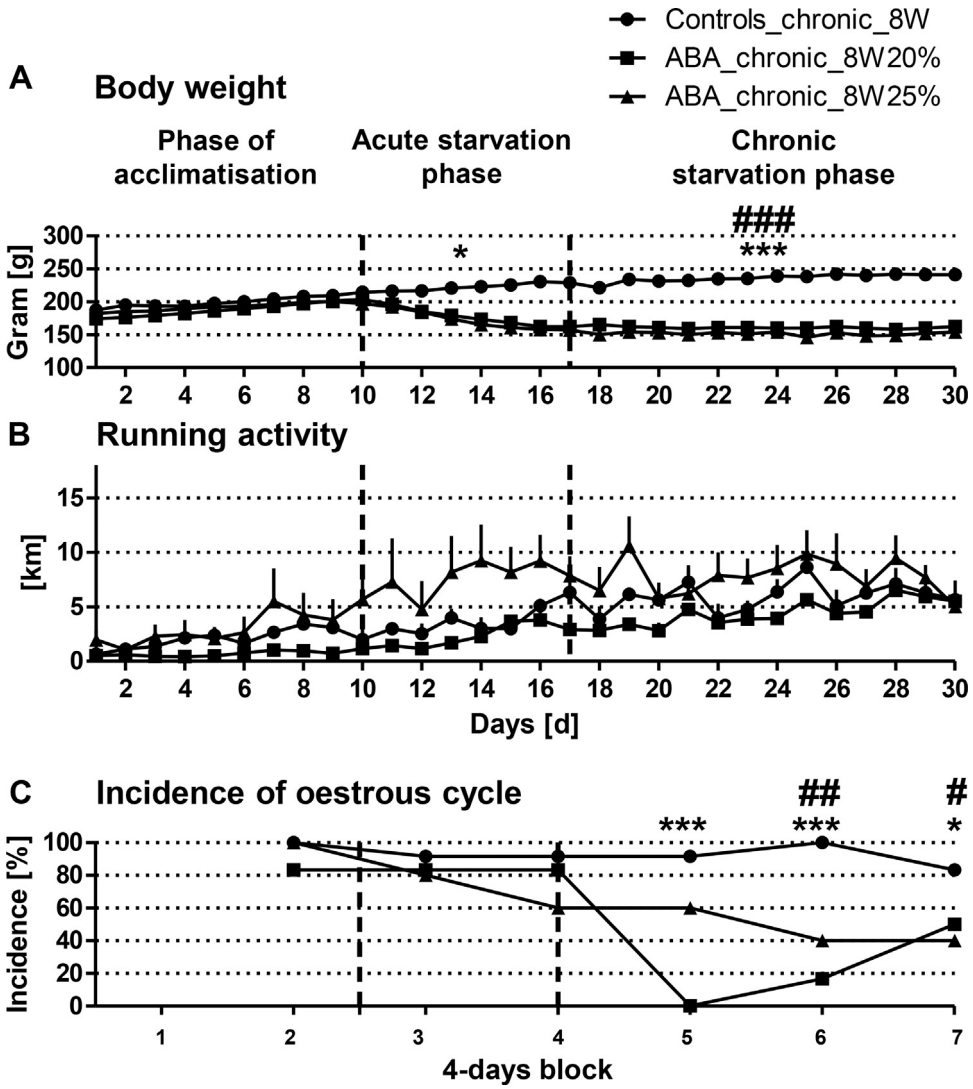

Fig. 4. The consequences of the 20% and 25% extent of starvation in 8-week-old ABA rodents and adequate controls. The standardised body weight, running wheel activity and incidence of oestrous cycle in 20% (A, C, E) and 25% 8-week-old animals (B, D, F) with chronic starvation. (A–D) *Control vs. 20% ABA, $^{*}p \leq 0.05$, $^{***}p \leq 0.001$, #Control vs. 25% ABA, $\#p \leq 0.05$, ## $p \leq 0.01$, ### $p \leq 0.001$, two-way ANOVA with repeated measures. (E-F) Chi-square tests comparing ABA and controls.

One important advantage of our modified chronic starvation model is the precise control of variations in body weight. This not only lets the experimenter choose and steer the experiment more accurately but also allows the model to be regarded as being milder with respect to physical stress to the rats, as over-starvation is prevented. Until now, none of the ABA rats had to be sacrificed because of starvation-related symptoms in our lab. In the original model, it was possible that the rats died or had to be euthanised, especially when they were also running during the short feeding period (Routtenberg and Kuznesof, 1967). Routtenberg and Kuznesof showed that all the rats that received 30 min/day food access died after an average of 13.5 days of starvation, and 2 animals died after 8 days (Routtenberg and Kuznesof, 1967). The fixed individual target weights of 25% body weight loss (controlled starvation) make inter-individual results more comparable, while in the other acute ABA models, there are differences in starvation extents or group-derived amounts of food only. Drug treatment in the modified ABA protocol can thus be easily performed with the help of osmotic mini-pumps (Alzet, Durect. Corp., USA), as drug dosage should be weight adapted.

One disadvantage of this ABA protocol is that we also had to isolate the rats in individual cages to individualise feeding and RWA measurements. This represents a social stress factor, which, as mentioned above, can influence behaviour and even permanent neuronal circuit changes (Deacon, 2006). However, by comparing with the controls of the same condition, this factor should be counterbalanced and not become a major limitation factor.

Additionally, by varying the duration of starvation, it is possible to analyse different duration stages of the illness and determine if starvation-induced effects are dependent on acute or chronic starvation. Our group already used these two stages of our modified ABA model to analyse the neurobiological consequences of two different starvation stages in parallel. We showed lower oestradiol and leptin plasma levels and showed the impaired recognition memory in ABA versus control animals to be more pronounced following chronic starvation (Paulukat et al., 2016). Oestradiol reduction was directly correlated to memory impairment, compatible with (but not proving) a causal role of oestrogen for certain forms of learning in ABA animals. This had been shown in ovariectomised rodents (Luine, 2008) and in patients with AN (Buehren et al., 2011). Lee et al. showed a reduction in oestrogen receptor β mRNA expression in the amygdala of ABA animals (two bouts of acute ABA) after one week of refeeding, making it even more interesting to analyse

the role of oestrogen and its receptors in starvation processes (Lee and Kinzig, 2017).

Furthermore, paralleling the human studies of brain volume loss in AN (Seitz et al., 2014), we also demonstrated brain volume loss in ABA animals (Frintrop et al., 2017). To determine the underlying mechanisms of this brain volume loss, we analysed cell count and morphology and for the first time found a loss of GFAP-positive astrocytes as well as a reduction of their surface area in the grey and white matter of chronic ABA animals. The reduced GFAP mRNA in the cerebral cortex and corpus callosum of these ABA rodents further corroborated these findings (Frintrop et al., 2017). These alterations of GFAP-positive astrocytes and surface areas were, however, not found after acute starvation, making these changes dependent on chronic hunger periods.

Future studies could further extend our model by introducing even longer chronic starvation phases to mimic long-term chronic severe and enduring eating disorder patients (SEED, Treasure et al., 2015), who are believed to suffer from neuro-progressive brain changes affecting cognition and behaviour. Additionally, refeeding regimes should be incorporated to learn more about the long-term consequences and reversibility of brain and behavioural changes in patients with AN. Together, the traditional ABA model has previously been shown to also work in mice. It would be a valuable addition to translate our chronic rat model also to mice. We would then be able to combine our modified ABA model with the possibility to use transgenic and knock-out mice to further unravel the underlying pathophysiology of AN.

5. Conclusion

To conclude, our modified chronic ABA protocol is effective, feasible, and harm-reducing, and it mimics several important aspects of human AN in an animal model. It allows the study of chronic starvation in a controlled manner and could become an important tool in understanding the underlying pathobiology of AN.

Acknowledgements and funding

We would like to acknowledge the support of Mareike Schulz, Pascal Paschenda and Dr. Kira Scherer at the Institute for Laboratory Animal Science; Helga Helten, Petra Ibold and Uta Zahn at the Institute of Neuroanatomy; and Dr. Cornelia Exner in the Department of Animal Physiology (Philipps-University Marburg, Marburg). This research was funded by the University Hospital Aachen, RWTH Aachen University, Germany (START 108/12).

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